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D This Application gains priority from U.S. Provisional Application Serial No. 60/102,413 filed September 30, 1998, entitled "Intein Mediated Peptide Ligation."

A marked-up version of page 1 of the specification showing the amendments made is attached, as well as a clean copy of page 1 of the specification. 37 C.F.R. §1.121(c)(1)(ii).

### IN THE CLAIMS

Please cancel claims 3, 14, 20, 31, 32, 59 and 61.

Please amend claims 1, 2, 4-9, 16, 17, 24, 34, 35, 40-42 and add new claim 62 as follows:

- D<sup>2</sup>
1. A method for ligating a first target protein with a second target protein, the method comprising the steps of:
    - (a) expressing in a host cell, a first fusion protein comprising the first target protein fused to an Mth RIR1 intein or modification thereof having N-terminal cleavage activity wherein the fusion protein is expressed from a first plasmid;
    - (b) contacting the fusion protein of step (a) with a thiol reagent for inducing cleavage of the intein to produce a C-terminal thioester on the first target protein; and
    - (c) combining in a mixture for permitting ligation, the C-terminal thioester on the first target protein and a thioester reactive N-terminal amino acid on the second target protein.

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D<sup>2</sup>

2. The method of claim 1, wherein the first plasmid further comprises at least one nucleic acid sequence that encodes at least one first modified *Mth* RIR1 intein having N-terminal cleavage activity and wherein said second target protein of step (c) is generated from a second plasmid which further comprises at least one nucleic acid sequence that encodes at least one second intein having C-terminal activity.

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4. The method of claim 2, wherein said first modified *Mth* RIR1 intein is selected from the group consisting of a Pro<sup>-1</sup> to Ala mutant intein, a Pro<sup>-1</sup> to Gly mutant intein, and a Pro<sup>-1</sup> - Asn<sup>134</sup> to Gly-Ala mutant intein, and wherein said second intein is selected from the group consisting of a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ser mutant intein and a Pro<sup>-1</sup> - Cys<sup>1</sup> to Gly-Ala mutant intein.

D<sup>3</sup>

5. The method of claim 2, wherein said first plasmid is selected from the group consisting of pMRB8A, pMRB8G1 and pMRB10G, and wherein said second plasmid is selected from the group consisting of pMRB9GS, pMRB9GA and pBRL-A.

6. The method of claim 2, wherein said first target protein of step (b) is generated by thiol reagent-induced cleavage of said first modified *Mth* RIR1 intein and said second target protein of step (c) is generated by temperature and/or pH induced cleavage of said second intein.

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D<sup>3</sup> Cont 7. The method of claim 1, wherein the thioester reactive N-terminal amino acid of step (c) is a cysteine or selenocysteine amino acid.

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8. A method for fusion of a first and a second target protein, the method comprising the steps of:

- D<sup>4</sup>
- (a) expressing in a cell (i) a first plasmid encoding at least a first target protein, and at least one first intein or modification thereof, wherein the expressed intein or modification thereof is capable of thiol induced cleavage to produce a C-terminal thioester for the first target protein; and (ii) a second plasmid encoding at least one second target protein and at least one second intein or modification thereof, the expressed second intein or modification thereof having C-terminal cleavage activity to produce an N-terminal cysteine or selenocysteine on the second protein; and
  - (b) ligating the C-terminal thioester on the first expressed target protein with the N-terminal cysteine or selenocysteine on the second expressed target protein to form a fusion protein.
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D<sup>5</sup> 9. The method of claim 8, wherein step (a) further comprises purifying said first target protein and said second target protein.

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D<sup>6</sup> 16. A method for cyclic fusion of a target protein, said method comprising the steps of:

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- (a) expressing a plasmid encoding (i) a first intein or modification thereof having N-terminal cleavage activity fused to the target protein; and (ii) a second intein or modification thereof having C-terminal cleavage activity, wherein said first intein is capable of thiol reagent-induced cleavage to produce a thioester at the C-terminal of the target protein and wherein the second intein is capable of cleavage to produce a cysteine or selenocysteine at the N-terminal of the target protein;
- (b) adding a thiol reagent; and
- (c) ligating the N-terminus of said target protein to the C-terminus of said target protein to produce a cyclic protein.

17. A method for polymerizing a plurality of target proteins in a preparation, said method comprising the steps of:

- Db
- (a) expressing from a plasmid in a host cell, a fusion protein, the fusion protein comprising a target protein and a first intein or modification thereof located at one end of the protein, and a second intein or modification thereof located at the second end of the target protein, wherein the first intein or modification thereof is capable of thiol reagent-induced cleavage to produce a thioester at the C-terminal of the target protein; and a second intein or modification thereof being capable of C-terminal cleavage activity to produce a cysteine or selenocysteine at the N-terminal of the target protein; and
  - (b) permitting intermolecular ligation between the C-terminal thioester on one target protein with an N-terminal cysteine

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*D6*  
or selenocysteine on a second target protein in the preparation.

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*D7*  
24. A fusion protein comprising an intein or modification thereof fused to a protein, wherein the intein or modification thereof is capable of pH or temperature induced cleavage from the protein, the protein after cleavage having an N-terminal cysteine or selenocysteine.

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*D8*  
34. The method of claim 1, further comprising:  
replacing in the first intein, a terminal proline residue with an alanine residue, the alanine residue having an N-terminal position with respect to a first amino acid of the intein.

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35. The method of claim 1, further comprising:  
replacing a C-terminal asparagine or cysteine of the intein with an alanine.

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*D9*  
40. A method for ligating a first protein target to a second target protein, comprising the steps of:

- (a) expressing by means of one or more plasmid, a first and a second protein, wherein the first protein comprises the first target protein and at least one first intein or modification thereof, and the second protein comprises the second target protein and optionally at least one second intein or modification thereof;
- (b) cleaving the first fusion protein in the presence of a nucleophilic reagent so as to provide the first target protein having a C-terminal thioester and either expressing the

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second protein having an N-terminal cysteine or selenocysteine or cleaving the second protein fused to the at least one intein or modification thereof to produce an N-terminal cysteine or selenocysteine; and

- (c) ligating the C-terminal thioester on the first target protein to the N-terminal cysteine or selenocysteine on the second target protein to form the protein product.

41. A method according to claim 40, wherein the step of cleaving further comprises separating the first and second target proteins from the cleaved one or more inteins.

42. A method for obtaining a protein product formed from two target proteins, said method comprising the steps of:

- D9  
cont
- (a) generating by *in vivo* synthesis, a first target protein fused to at least one first intein and a second target protein; the second protein having an N-terminal cysteine or selenocysteine, or optionally fused to a second intein for cleavage to form an N-terminal cysteine or selenocysteine
- (b) cleaving the first target protein from at least one first intein so as to form a C-terminal thioester; and optionally cleaving the second target protein from at least one second intein so as to produce an N-terminal cysteine or selenocysteine; and
- (c) ligating the C-terminal thioester on the first target protein with the N-terminal cysteine or selenocysteine on the second target protein to form the protein product.
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